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Experimental paper

Nitroglycerin attenuates vasoconstriction of HBOC-201 during hemorrhagic shock resuscitation☆,☆☆

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ABSTRACT

Background: Vasoconstriction, an inherent property of Hemoglobin Based Oxygen Carriers (HBOC) potentially due to nitric oxide (NO) scavenging, may increase cardiovascular complications in HBOC resuscitated trauma patients. The purpose of this study was to determine if co-administration of a weak NO donor, intravenous nitroglycerin (NTG), with HBOC-201 during resuscitation from hemorrhagic shock could safely attenuate HBOC-201 vasoconstriction.

Methods and results: Hemorrhagic shock was induced in 44 swine randomized to receive fluid resuscitation with HBOC, HBOC + NTG10 mcg/kg/min, HBOC + NTG20 mcg/kg/min, HBOC + NTG40 mcg/kg/min, Hetastarch (HES), HES + NTG20 mcg/kg/min, NTG20 mcg/kg/min and Lactated Ringers (LR). HBOC resuscitation from hemorrhagic shock increased mean arterial pressure (MAP = 94 ± 33 mmHg), mean pulmonary artery pressure (MPAP = 29 ± 11 mmHg) and systemic vascular resistance (SVR = 2684 ± 871 dyn s/cm^5) in comparison to HES. Co-administration of NTG during HBOC resuscitation attenuated vasoconstriction with HBOC + 40 mcg/kg/min demonstrating the most robust reduction in vasoconstriction (MAP = 59 ± 23 mmHg, MPAP = 18 ± 7 mmHg, and SVR = 1827 ± 511 dyn s/cm^5), although the effects were transient. Co-administration of NTG with HBOC did not alter base deficit, lactate, methemoglobin levels, nor cause profound hypotension during resuscitation.

Conclusion: Nitroglycerin attenuates vasoconstrictive properties of HBOC when co-administered during resuscitation in this swine model of hemorrhagic shock. Translational survival studies are required to determine if this strategy of attenuation of the vasoconstriction of HBOC-201 reduces cardiovascular complications and improves outcome with HBOC fluid resuscitation for hemorrhagic shock.

Hemoglobin Based Oxygen Carriers (HBOC) hold great promise for the treatment of hemorrhagic shock. However, HBOC have failed to improve survival in trauma clinical trials.[1] Vasoconstriction, an inherent property of HBOC, may increase cardiovascular complications in trauma patients resuscitated with HBOC and this may explain in part why HBOC, in their current form, fail to improve survival. These concerns about the vasoconstrictive properties of HBOC have clouded discussions about further human trials of HBOC.[2]

HBOC scavenge nitric oxide (NO) and the reduction of NO may induce vasoconstriction.[3,4] Nitroglycerin (NTG) is a weak NO donor that causes vasodilatation.[5,6] Nitroglycerin is also commonly used for the treatment of acute cardiovascular disease.[7] The effects of administering nitroglycerin during HBOC fluid resuscitation from hemorrhagic shock are not well studied, but may offer a practical agent for attenuating the potential vasoconstrictive complications of HBOC.

The purpose of this study was to examine the cardiovascular and metabolic effects of co-administration of intravenous NTG with HBOC-201 during resuscitation from hemorrhagic shock in a swine model. HBOC-201 is an ultra-purified glutaraldehyde-polymerized bovine hemoglobin solution that improves the delivery of oxygen to tissues, is stable between 2 and 30 °C for at least 3 years, requires no reconstitution, and could be readily available for use by emer-

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14. ABSTRACT

Background: Vasoconstriction, an inherent property of Hemoglobin Based Oxygen Carriers (HBOC) potentially due to nitric oxide (NO) scavenging, may increase cardiovascular complications in HBOC resuscitated trauma patients. The purpose of this study was to determine if co-administration of a weak NO donor. intravenous nitroglycerin (NTG), with HBOC-201 during resuscitation from hemorrhagic shock could safely attenuate HBOC-201 vasoconstriction. Methods and results: Hemorrhagic shock was induced in 44 swine randomized to receive fluid resuscitation with HBOC, HBOC+NTG10mcgjkg/min, HBOC+NTG20mcg/kg/min, HBOC+NTG40mcg/kg/min Hetastarch (HES). HES + NTG20 meg/kg/min, NTG20 meg/kg/min and Lactated Ringers (LR). HBOC resuscitation from hemorrhagic shock increased mean arterial pressure (MAP"" 94 ? 33 mmHg) mean pulmonary artery pressure (MPAP .. 29? 11 mmHg) and systemic vascular resistance (SVR==2684?871 dyns/cm5) in comparison to HES. Co-administration of NTG during HBOC resuscitation attenuated vasoconstriction with HBOC + 40 meg/kg/min demonstrating the most robust reduction in vasoconstriction (MAP .. 59?23 mmHg. MPAPa 18?7mmHg, and SVR"" 1827?511 dynsfcm5) although the effects were transient. Co-administration of NTG with HBOC did not alter base deficit lactate, methemoglobin levels, nor cause profound hypotension during resuscitation. Conclusion: Nitroglycerin attenuates vasoconstrictive properties of HBOC when co-administered during resuscitation in this swine model of hemorrhagic shock. Translational survival studies are required to determine if this strategy of attenuation of the vasoconstriction of HBOC-201 reduces cardiovascular complications and improves outcome with HBOC fluid resuscitation for hemorrhagic shock.

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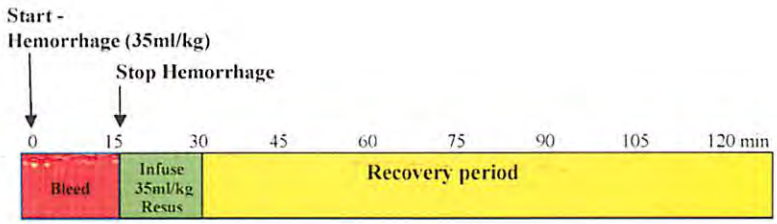

Fig. 1. Experimental design: 35 ml/kg bleed over 15 min followed by an equivalent volume of test solution infused over 15 min during the fluid resuscitation (Resus) phase. Cardiovascular and metabolic values were monitored throughout the protocol.

gency medical services and military medics in the prehospital or battlefield setting.[8–10] We hypothesized that co-administration of nitroglycerin with HBOC during resuscitation from hemorrhagic shock attenuates the vasoconstrictive properties of HBOC-201 in an experimental model of hemorrhagic shock.

1. Methods

The study was approved by the University of North Carolina's Institute for Animal Care and Use Committee. The experiments reported herein were conducted in compliance with the Animal Welfare Act and in accordance with the principles set forth in the "Guide for the Care and Use of Laboratory Animals", Institute of Laboratory Animals Resources, National Research Council, National Academy Press, 1996. Fifty male farm bred swine (29.2 ± 4.3 kg) were anesthetized with ketamine (500 mg IM) and titrated intravenous propofol (150–400 mcg/kg/h) anesthesia and intubated for mechanical ventilation. The swine were instrumented for placement of a Swan-Ganz catheter and bilateral femoral arterial and venous catheters. A midline incision was made from the xiphoid to symphus pubis and the mesenteric artery was identified and a Doppler flow probe was placed around the vessel. A suprapubic catheter was placed in the urinary bladder and all incisions were closed with sutures. Upon completion of the surgical preparations, a baseline period of approximately 15–30 min allowed for the stabilization of cardiopulmonary physiology between groups before the acute insult. At the end of the baseline period, hemorrhagic shock was induced by withdrawal of 35 ml/kg of blood from the femoral arterial catheter at a constant rate over a 15 min period. At the end of blood withdrawal, the swine were block randomized to one of eight groups: HBOC-201 alone (HBOC, $N=7$), HBOC + NTG10 mcg/kg/min (HBOC + NTG10, $N=7$), HBOC + NTG20 mcg/kg/min (HBOC + NTG20, $N=7$), HBOC + NTG40 mcg/kg/min (HBOC + NTG40, $N=7$), Hetastarch 6% (HES) ($N=6$), HES + NTG20 mcg/kg (HES + NTG20, $N=4$), NTG20 mcg/kg alone (NTG20, $N=4$) and Lactated Ringers (LR) ($N=2$). Investigators were blinded to therapeutic interventions by a drape that covered the infusion lines and separated them from the technician who infused the solutions. Each group received a volume of 35 ml/kg of test fluid administered at a constant rate over a 15 min period during the fluid resuscitation phase. Cardiovascular and blood gas monitoring was continued for an additional 90 min after completion of fluid resuscitation. Fig. 1 summarizes the protocol timeline. Two LR experiments were performed to define the response of the model to crystalloid resuscitation. Hetastarch was used as the control solution because it has an oncotic pressure similar to HBOC-201.

1.1. Statistics

All values were reported as means and standard deviations. Cardiovascular and metabolic parameters were compared between groups at baseline (−5 to 0 min), hemorrhage (0–15 min) fluid resuscitation (16–30 min) and during recovery (31–45 min, 46–60 min, 61–90 min and 91–120 min) using a mixed model to account for the correlated nature of the repeated measurements within the same animal. A non-linear regression model was used to identify the point in time following the hemorrhage period when specific parameters (i.e., MAP, MPAP) reached a plateau; that is, the duration of the treatment effect. p-Values of 0.05 (two-sided) were considered statistically significant.

2. Results

Forty-four swine completed the protocol while six were prospectively excluded because of anesthesia and surgical complications. Surgical preparation time averaged 122 ± 14 min and did not differ between groups. All cardiovascular and metabolic parameters were similar between groups during baseline before blood withdrawal.

Withdrawal of 35 ml/kg of arterial blood over 15 min led to hemorrhagic shock (MAP 22 ± 18 mmHg) in all groups combined. There were no differences between groups in cardiovascular and metabolic parameters during hemorrhage (data not shown). All animals that received HBOC, HBOC + NTG, HES + NTG20 or LR during fluid resuscitation survived to the end of the protocol (120 min) while the HES ($N=5/6$, mean survival 108 ± 24) and NTG20 groups ($N=2/4$, mean survival 78 ± 48 min) survived to the end of the protocol. The HBOC alone group had the greatest rise in MAP during fluid resuscitation and the NTG alone group had the least rise in MAP. Fig. 2 shows the changes in MAP with fluid resuscitation for all treatment groups. Table 1 provides values for the MAP, heart rate (HR), cardiac output (CO), mean pulmonary artery pressure (MPAP) and systemic vascular resistance (SVR) during baseline, fluid resuscitation and recovery. All doses of NTG co-administered with HBOC

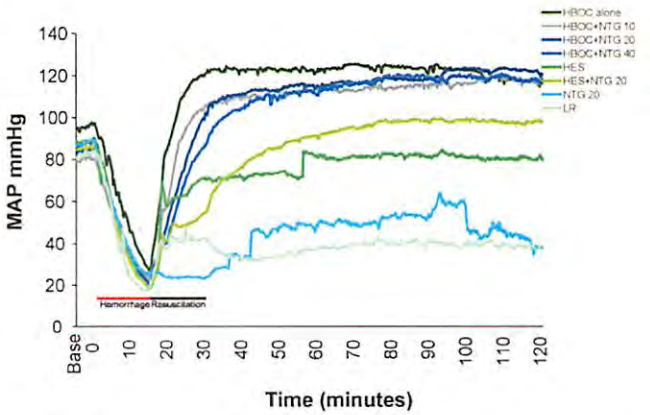

Fig. 2. Mean arterial pressure (MAP) during hemorrhage (35 ml/kg over 15 min) followed by fluid resuscitation (35 ml/kg over 15 min). Resuscitation fluids include Hemoglobin Based Oxygen Carrier-201 alone (HBOC), HBOC and co-administration of intravenous nitroglycerin (NTG) 10 mcg/kg/min (HBOC + NTG10), HBOC + NTG20 mcg/kg/min (HBOC + NTG20), HBOC + NTG40 mcg/kg/min (HBOC + NTG40), 6% Hetastarch (HES), HES + NTG20 mcg/kg/min (HES + NTG20), NTG20 mcg/kg/min alone (NTG20) and Lactated Ringers (LR).

Table 1
Cardiovascular parameters during baseline, fluid resuscitation (resuscitation) and times during early and late recovery. HBOC = Hemoglobin Based Oxygen Carrier alone, HBOC-NTG10 = HBOC co-administered with NTG10 mcg/kg/min, HBOC-NTG20 = HBOC co-administered with NTG20 mcg/kg/min, HBOC-NTG40 = HBOC co-administered with NTG40 mcg/kg/min, HES = Hetastarch, HES-NTG20 = HES co-administered with NTG20 mcg/kg/min, NTG = NTG20 mcg/kg/min alone. Values reported as means ± standard deviations. Statistical significance in comparison to the HBOC alone group.

Intervention	HBOC	HBOC-NTG10	HBOC-NTG20	HBOC-NTG40	HES	HES-NTG20	NTG20
Baseline							
MAP (mmHg)	96 ± 10	80 ± 9	84 ± 14	88 ± 11	88 ± 11	86 ± 12	88 ± 6
HR (bpm)	97 ± 12	89 ± 13	98 ± 12	91 ± 9	94 ± 22	90 ± 11	98 ± 12
CO (L/min)	4.32 ± 0.78	3.51 ± 0.57	3.97 ± 0.88	3.61 ± 0.54	3.35 ± 0.48	3.79 ± 0.78	3.97 ± 0.55
MPAP (mmHg)	20 ± 3	16 ± 3	17 ± 3	17 ± 2	21 ± 5	17 ± 4	16 ± 2
SVR (dyn s/cm^5)	1738 ± 404	1760 ± 548	1666 ± 616	1944 ± 438	2017 ± 383	1696 ± 295	1726 ± 249
Resuscitation							
MAP (mmHg)	94 ± 33	77 ± 27 $p = 0.011$	66 ± 28 $p < 0.0001$	59 ± 23 $p < 0.0001$	59 ± 23 $p < 0.0001$	44 ± 14 $p < 0.0001$	24 ± 9 $p < 0.0001$
HR (bpm)	131 ± 43	134 ± 46	133 ± 41	132 ± 40	143 ± 40	137 ± 40	168 ± 42
CO (L/min)	3.11 ± 0.92	3.42 ± 0.8 3	3.56 ± 1.58	3.25 ± 1.15	4.23 ± 1.86	3.87 ± 1.33	0.83 ± 0.47 $p < 0.0001$
MPAP (mmHg)	29 ± 11	20 ± 7 $p < 0.0001$	18 ± 7 $p < 0.0001$	18 ± 7 $p < 0.0001$	19 ± 6 $p < 0.0001$	17 ± 3 $p < 0.0001$	11 ± 3 $p < 0.0001$
SVR (dyn s/cm^5)	2684 ± 871	1934 ± 402 $p < 0.0226$	1786 ± 795 $p < 0.0069$	1827 ± 511 $p < 0.0016$	1252 ± 292 $p = 0.0004$	810 ± 162 $p < 0.0001$	2242 ± 517
Recovery (30–45 min)							
MAP (mmHg)	123 ± 13	109 ± 8	110 ± 12	102 ± 13 $p = 0.001$	72 ± 20 $p < 0.0001$	73 ± 16 $p < 0.0001$	32 ± 19 $p < 0.0001$
HR (bpm)	103 ± 9	99 ± 18	108 ± 27	101 ± 16	115 ± 27	111 ± 12	180 ± 55 $p = 0.001$
CO (L/min)	3.85 ± 0.96	3.83 ± 0.52	4.50 ± 1.81	3.72 ± 0.65	4.37 ± 1.44	5.57 ± 1.72	1.47 ± 0.71 $p = 0.0011$
MPAP (mmHg)	40 ± 6	30 ± 5 $p = 0.0007$	32 ± 4 $p = 0.0029$	30 ± 7 $p = 0.0005$	20 ± 3 $p < 0.0001$	21 ± 3 $p < 0.0001$	12 ± 4 $p < 0.0001$
SVR (dyn s/cm^5)	2480 ± 717	2138 ± 429	2028 ± 744	2129 ± 568	1256 ± 317 $p = 0.0003$	946 ± 151 $p < 0.0001$	2008 ± 114
Recovery (60–90 min)							
MAP (mmHg)	124 ± 9	114 ± 7	117 ± 9	117 ± 12	82 ± 8 $p < 0.0001$	96 ± 20 $p < 0.0001$	51 ± 16 $p < 0.0001$
HR (bpm)	99 ± 12	87 ± 10	95 ± 22	89 ± 11	120 ± 34	124 ± 6	229 ± 9 $p < 0.0001$
CO (L/min)	3.69 ± 1.02	3.50 ± 0.62	4.02 ± 1.67	3.47 ± 0.59	4.84 ± 1.43	6.08 ± 1.22 $p = 0.0078$	1.72 ± 0.68 $p = 0.042$
MPAP (mmHg)	33 ± 6	26 ± 4 $p = 0.0018$	28 ± 4 $p = 0.0281$	26 ± 5 $p = 0.0032$	19 ± 3 $p < 0.0001$	21 ± 3 $p < 0.0001$	13 ± 2 $p < 0.0001$
SVR (dyn s/cm^5)	2679 ± 872	2519 ± 584	2388 ± 793	2557 ± 679	1374 ± 545 $p = 0.0023$	1126 ± 278 $p = 0.0009$	2102 ± 81

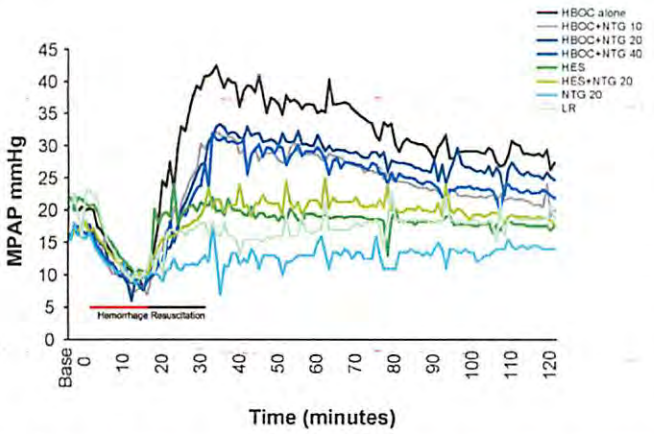

Fig. 3. Mean pulmonary artery pressure (MPAP) during hemorrhage (35 ml/kg over 15 min) followed by fluid resuscitation (35 ml/kg over 15 min). Resuscitation fluids include Hemoglobin Based Oxygen Carrier-201 alone (HBOC), HBOC and co-administration of intravenous nitroglycerin (NTG) 10 mcg/kg/min (HBOC + NTG10), HBOC + NTG20 mcg/kg/min (HBOC + NTG20), HBOC + NTG40 mcg/kg/min (HBOC + NTG40), 6% Hetastarch (HES), HES + NTG20 mcg/kg/min (HES + NTG20), NTG20 mcg/kg/min alone (NTG20) and Lactated Ringers (LR).

attenuated MAP, MPAP and SVR during fluid resuscitation. The HBOC + NTG40 was the only treatment group that demonstrated persistent attenuation of MAP when compared to the HBOC alone group during the late recovery period (Table 1). In contrast, all HBOC + NTG groups had attenuation in MPAP that persisted in the late recovery period after fluid resuscitation (Table 1 and Fig. 3). SVR was attenuated in the HBOC + NTG10, HBOC + NTG20 and HBOC + NTG40 groups compared to HBOC alone during fluid resuscitation (Table 1 and Fig. 4). However, these differences resolved during the recovery period. There were no significant differences in CO or HR in the HBOC + NTG10, HBOC + NTG20 and HBOC + NTG40 groups compared to HBOC alone group. However, there were differences in CO between the HES, NTG20 and HBOC alone groups throughout recovery.

The duration of HBOC alone vasoconstriction persisted for 23.8 min after fluid resuscitation. Co-administration of NTG attenuated the HBOC-associated vasoconstriction for 2.7 (HBOC + NTG10), 7.4 (HBOC + NTG20) and 11.5 (HBOC + NTG40) minutes respectively after fluid resuscitation. A similar pattern of vasoconstriction and attenuation were apparent for the pulmonary system. The duration of HBOC alone MPAP vasoconstriction persisted for 23.9 min after fluid resuscitation. Co-administration of NTG attenuated the HBOC-associated vasoconstriction in MPAP for 2.2 (HBOC + NTG10), 5.5 (HBOC + NTG20) and 6.0 (HBOC + NTG40) minutes respectively after fluid resuscitation.

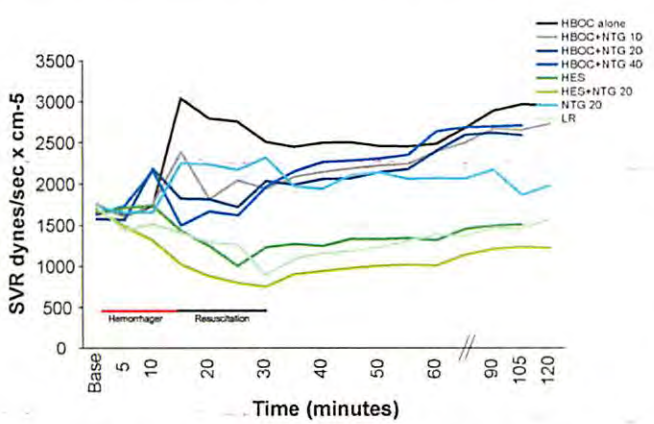

Fig. 4. Mean systemic vascular resistance (SVR) during hemorrhage (35 ml/kg over 15 min) followed by fluid resuscitation (35 ml/kg over 15 min). Resuscitation fluids include Hemoglobin Based Oxygen Carrier-201 alone (HBOC), HBOC and co-administration of intravenous nitroglycerin (NTG) 10 mcg/kg/min (HBOC + NTG10), HBOC + NTG20 mcg/kg/min (HBOC + NTG20), HBOC + NTG40 mcg/kg/min (HBOC + NTG40), 6% Hetastarch (HES), HES + NTG20 mcg/kg/min (HES + NTG20), NTG20 mcg/kg/min alone (NTG20) and Lactated Ringers (LR).

The average mesenteric arterial blood flow (percent of baseline) for all groups decreased to 30 ± 10% of baseline at the end of hemorrhage. There were no differences in mesenteric arterial blood flow between groups during and after fluid resuscitation except when the HBOC alone (118 ± 15%) was compared to the NTG20 (39 ± 30%) group during fluid resuscitation. Mesenteric arterial blood flow remained elevated above baseline in all groups except the NTG alone group after fluid resuscitation.

Base deficit and lactate values increased significantly above baseline in all groups during fluid resuscitation (Table 2). There was no difference in base deficit and lactate levels when comparing fluid resuscitation with HBOC + NTG groups to the HBOC alone group. Base deficit and lactate levels in the HES group were significantly increased compared to groups with HBOC as a component of the resuscitation fluid. Methemoglobin (MetHB) values remained at baseline for all non-HBOC containing fluids (HES, HES + NTG, NTG and LR groups) during fluid resuscitation and recovery. There was an increase in MetHb levels from baseline in all HBOC containing fluids groups during fluid resuscitation and the elevation remained throughout recovery. There was no difference in MetHb levels between the HBOC alone group and the HBOC groups containing NTG.

3. Discussion

Infusion of HBOC-201 during hemorrhagic shock increased vasoconstriction as was apparent by elevated blood pressure, pulmonary artery pressure and systemic vascular resistance. Nitroglycerin co-administered with HBOC reduced vasoconstriction, although the response was transient. The short half life of intravenous nitroglycerin,[11] relative to the longer duration of HBOC vasoconstriction, may explain the transient attenuation of vasoconstriction associated with co-administration of NTG. Colleagues at Brown University (Dr. Hai), University of Alabama (Dr. Kirby), Naval Medical Research Center (Dr. Freilich) and Commonwealth of Virginia (Dr. Pittman) have shown similar inhibition of HBOC-201 vasoconstriction with NTG and the NO donor sodium nitrite using *in vitro* vascular ring and *in vivo* mouse and swine hemorrhagic shock models (unpublished, personal communication). Future studies are being designed to determine whether repeated doses of NTG can provide prolonged attenuation of vasoconstriction associated with HBOC resuscitation.

The study design allowed the evaluation of whether intravenous nitroglycerin attenuates the vasoconstrictive effects of HBOC-201 during resuscitation from hemorrhagic shock. Unlike our earlier work of hemorrhagic shock, where we utilized a translational model of hemorrhagic shock in large mammals (swine),[12,13] the modified Wigger's model[14] used in this study provided a more precise method for evaluating the cardiovascular and metabolic changes. The modified Wigger's model allowed control of the confounding variable of volume status[15] during resuscitation since the volume of blood withdrawn during hemorrhage was replaced with the same volume of resuscitation fluid over the same time period in each group during resuscitation. Hetastarch was chosen as the control solution for comparison because its oncotic properties were similar to HBOC, yet it has minimal vasoconstrictive properties.[16,17]

Hemoglobin outside of erythrocytes (cell free hemoglobin) interacts with nitric oxide and is associated with vasoconstriction.[18–22] A similar interaction occurs with HBOC and previous work has shown that a variety of NO donors can

Table 2
Metabolic parameters during baseline, fluid resuscitation (resuscitation) and recovery. HBOC = Hemoglobin Based Oxygen Carrier alone, HBOC-NTG10 = HBOC co-administered with NTG10 mcg/kg/min, HBOC-NTG20 = HBOC co-administered with NTG20 mcg/kg/min, HBOC-NTG40 = HBOC co-administered with NTG40 mcg/kg/min, HES = Hetastarch, HES-NTG20 = HES co-administered with NTG20 mcg/kg/min, NTG = NTG20 mcg/kg/min alone. Values reported as means ± standard deviations. Statistical significance in comparison to the HBOC alone group except for MetHb where significance reported in comparison to Hetastarch.

Intervention	HBOC	HBOC-NTG10	HBOC-NTG20	HBOC-NTG40	HES	HES-NTG20	NTG20
Baseline							
Base deficit	5.8 ± 2.0	5.7 ± 2.0	6.6 ± 1.1	5.1 ± 1.3	3.6 ± 2.3	4.2 ± 1.0	8.0 ± 6.4
Lactate	1.4 ± 0.6	1.1 ± 0.4	1.7 ± 0.9	1.2 ± 0.5	1.7 ± 0.5	2.1 ± 0.5	2.3 ± 1.6
Hemoglobin	9.5 ± 0.7	9.2 ± 0.9	9.2 ± 0.5	9.2 ± 1.2	9.8 ± 1.2	9.4 ± 1.0	8.8 ± 0.5
Methemoglobin	1.8 ± 0.2	1.7 ± 0.2	1.8 ± 0.1	1.6 ± 0.2	1.7 ± 0.2	1.9 ± 0.4	1.8 ± 0.3
Resuscitation							
Base deficit	−0.5 ± 2.0	−1.2 ± 2.0	−1.8 ± 3.5	−2.7 ± 2.5	−3.8 ± 4.5 $p = 0.0145$	−2.8 ± 1.5	2.0 ± 5.8
Lactate	3.2 ± 0.7	4.3 ± 1.6	3.5 ± 1.3	4.4 ± 1.6	3.7 ± 1.0	4.3 ± 0.6	5.9 ± 1.3
Hemoglobin	9.8 ± 0.6	9.9 ± 0.8	9.9 ± 0.7	9.6 ± 0.9	5.6 ± 1.9 $p = 0.0207$	5.5 ± 1.8 $p = 0.0284$	9.1 ± 1.2
Methemoglobin	2.7 ± 0.4 $p = 0.002$	2.6 ± 0.5 $p = 0.025$	2.7 ± 0.3 $p = 0.008$	2.7 ± 0.4 $p = 0.0173$	1.5 ± 0.5	1.8 ± 0.4	1.8 ± 0.3
Recovery (30–60 min)							
Base deficit	3.1 ± 2.0	3.4 ± 2.0	3.2 ± 2.8	1.6 ± 2.0	−0.4 ± 4.0 $p = 0.0173$	1.1 ± 1.5	−4.4 ± 2.4 $p = 0.0109$
Lactate	2.6 ± 0.5	2.7 ± 1.1	3.5 ± 1.3	3.1 ± 1.8	3.5 ± 1.9	3.5 ± 0.4	7.7 ± 0.4 $p < 0.0001$
Hemoglobin	9.8 ± 0.6	9.0 ± 0.8	9.0 ± 0.8	9.1 ± 0.6	4.0 ± 1.2 $p < 0.0001$	3.3 ± 1.0 $p < 0.0001$	9.3 ± 1.6
Methemoglobin	3.7 ± 0.4 $p < 0.0001$	3.8 ± 0.4 $p < 0.0001$	3.8 ± 0.4 $p < 0.0001$	3.7 ± 0.4 $p < 0.0001$	1.8 ± 0.6	1.6 ± 0.4	1.9 ± 0.3
Recovery (60–90 min)							
Base deficit	5.5 ± 2.7	6.3 ± 1.3	6.6 ± 1.9	5.1 ± 1.6	2.7 ± 2.4	3.9 ± 1.1	−3.0 ± 1.2 $p < 0.0001$
Lactate	1.4 ± 0.4	1.3 ± 0.5	1.8 ± 0.8	1.6 ± 1.1	2.0 ± 0.6	2.6 ± 0.4 $p = 0.049$	7.1 ± 0.5 $p < 0.0001$
Hemoglobin	8.9 ± 0.3	8.7 ± 0.5	9.0 ± 0.8	9.0 ± 0.6	4.2 ± 1.4 $p < 0.0001$	3.0 ± 0.8 $p < 0.0001$	8.8 ± 0.4
Methemoglobin	4.5 ± 0.5 $p < 0.0001$	4.7 ± 0.6 $p < 0.0001$	4.6 ± 0.3 $p < 0.0001$	4.4 ± 0.4 $p < 0.0001$	1.9 ± 0.5	1.6 ± 0.4	1.9 ± 0.5
Recovery (90–120 min)							
Base deficit	6.4 ± 2.2	7.5 ± 1.2	8.0 ± 1.8	6.0 ± 1.8	4.1 ± 2.3	5.2 ± 1.1	−3.2 ± 3.0 $p < 0.0001$
Lactate	1.2 ± 0.3	0.9 ± 0.2	1.2 ± 0.5	1.0 ± 0.6	1.6 ± 0.6	1.8 ± 0.2	7.8 ± 1.6 $p < 0.0001$
Hemoglobin	8.9 ± 0.5	8.7 ± 0.5	8.8 ± 0.4	8.9 ± 0.7	4.3 ± 1.7 $p < 0.0001$	3.3 ± 1.0 $p < 0.0001$	8.2 ± .4
Methemoglobin	5.0 ± 0.5 $p < 0.0001$	5.3 ± 0.7 $p < 0.0001$	5.2 ± 0.4 $p < 0.0001$	4.9 ± 0.3 $p < 0.0001$	1.6 ± 0.4	1.6 ± 0.6	1.8 ± 0.6

attenuate HBOC vasoconstriction *in vitro*, *ex vivo* and *in vivo*.[23–29] Our results support the finding that the NO donor NTG attenuates HBOC vasoconstriction in the setting of hemorrhagic shock. Nitroglycerin reduces vascular resistance in small and large vessels by endothelial independent, NO mediated vasodilatation.[30,31] However, nitroglycerin does not eliminate these vascular effects during recovery and additional studies are required to determine whether increased dosing of NTG or repeated doses of NTG can eliminate the vasoconstriction of HBOC and whether these alterations have clinical significance. NTG alone did not induce a profound further drop in blood pressure nor vasodilatation during resuscitation from hemorrhagic shock even though endothelial independent vasodilatory properties are reportedly preserved during hemorrhagic shock.[32] These findings suggest that there may be a margin of safety when NTG is co-administered with HBOC during resuscitation from hemorrhagic shock. As expected, HBOC caused mild elevations in MetHb levels during resuscitation since the free hemoglobin present in HBOC auto-oxides in the circulation and forms methemaglobinemia.[33] The level of MetHb in this study was well below that which causes toxicity or reductions in oxygen delivery to tissues[33] and the addition of NTG to HBOC did not elevate MetHb levels further. Since NTG is used in clinical practice to reduce infarct size, morbidity and mortality from acute coronary ischemia, there may be fewer obstacles to regulatory approval of NTG versus experimental agents as a drug to co-administer with HBOC.[7,34]

HBOC reduced base deficit and lactate levels compared to the non-oxygen carrying Hetastarch solution during resuscitation from hemorrhagic shock in this large animal model. These findings are consistent with our earlier work and reports by others.[12,13,35] However, intravenous NTG, co-administered with HBOC-201 neither worsened nor improved base deficit or lactate levels during resuscitation compared to HBOC alone. These results indirectly suggest that the NTG induced attenuation of HBOC vasoconstriction does not compromise HBOC delivery of oxygen to tissues, although oxygen delivery was not directly evaluated in this study.

Mesenteric arterial blood flow was reduced during hemorrhagic shock. Mesenteric blood flow was restored during resuscitation in all groups except the NTG alone treatment group. Even though NO vasodilatation is more dependent on flow than pressure[36] it is likely that the failure of NTG to increase mesenteric blood flow during resuscitation represents the failure of NTG as a resuscitation fluid to sufficiently raise blood pressure during resuscitation versus a release of NO and vasodilatation of the mesenteric circulation. Unfortunately, mesenteric vascular tone was not measured in this study.

3.1. Limitations

The modified Wigger's[14] large animal model used in this study produced relatively mild shock, had no tissue injury, no continued bleeding during resuscitation and the survival time after resuscitation was relatively brief, so the results have limited translational value.[12,37] However, the study design allowed for the evaluation of the cardiovascular and metabolic changes when nitroglycerin was co-administered with HBOC-201 during resuscitation from hemorrhagic shock while controlling the confounding variable of volume status.[38] This mechanistic study was a necessary step to identify an agent that has the potential to attenuate HBOC-induced vasoconstriction. The results of this study provide a frame of reference for dosing information necessary for the further development of translational survival studies for NTG and HBOC administration during resuscitation from hemorrhagic shock.

The fixed doses of co-administered NTG used to attenuate HBOC vasoconstriction in this swine model are higher than those normally used in clinical practice.[11,39,40] The study design does not allow us to determine whether these high doses are species specific or a consequence of hemorrhagic shock. We did one pilot experiment with escalating doses of NTG in a euvolemic swine and did not observe a decrease in blood pressure until the dose reached 10 mcg/kg/min, but additional studies will be required to confirm this observation. In addition, dose escalation studies will be necessary to establish dose response curves in humans.

4. Conclusion

Nitroglycerin attenuated the vasoconstrictive properties of HBOC-201 without causing significant hypotension or clinically relevant methemaglobinemia when co-administered during resuscitation in a swine model of hemorrhagic shock. Translational survival studies are required to determine if this strategy of attenuation of the vasoconstriction of HBOC-201 reduces potential cardiovascular complications and improves outcomes with HBOC-201 fluid resuscitation for hemorrhagic shock.

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Conflict of interest statement

No conflict of interest.

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